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**Immunohistochemical Expression of Syndecan-1 in
Erosive Lichen Planus, Epithelial Dysplasia and Oral
Squamous Cell Carcinoma**

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Abstract

Background and Objective: syndecan-1, a member of a family of cell-surface proteoglycans, is known to bind with cell-to-cell and cells-to-interstitial matrix. The aim of the present study was to evaluate the syndecan-1 expression in erosive lichen planus, epithelial dysplasia and oral squamous cell carcinoma (OSCC).

Materials and methods: A total of 10 erosive lichen planus, 5 epithelial dysplasia and 20 OSCC were prepared for immunohistochemical staining of syndecan-1. The results were scored mild, moderate and severe based on the predominant staining intensity and characterization of membranous and cytoplasmic membrane staining.

Results: The results of staining intensity in OSCC in most of samples were mild (50%) or moderate (45%). The lichen planus in most cases had severe (50%) or moderate (40%) staining. As well as, in the dysplasia cases 4 samples (80%) had severe staining and one case (20%) showed moderate staining and the difference was statistically significant ($p = 0.03$). In more cases of OSCC (90%) both membranous and cytoplasmic expression was observed. Membranous staining was observed in 8 (80%) cases of lichen planus. The membranous staining was observed in all samples (100%) of dysplasia, and the difference was statistically significant ($p=0.00$).

Conclusion: The expression of syndecan-1 in OSCC was significantly less than lichen planus and dysplasia. The reduction of the expression of syndecan-1 in Lichen Planus (similar to what was seen in OSCC) may be a sign of a potential pre-malignant of lesion, but this requires further investigation.

Keywords: Syndecan-1, Erosive lichen planus, Epithelial dysplasia, Squamous cell carcinoma, Immunohistochemistry.

Introduction

In recent years significant advances have been achieved in molecular and genetic changes that provoke the development of cancer and biological behavior. These insights have led to the introduction of novel targeted therapies, revolutionizing the management of patients with advanced disease.¹

Oral lichen planus is a relatively common mucocutaneous lesion among middle-aged and elderly people. White patches, erythematous erosions, and ulcers may occur. The white lesions are not painful, but the erosions and ulcers are usually painful.² It might be controversial for pathologists to evaluate the risk of malignant transformation in lichen planus. Although

some of these lesions, especially erosive type are considered to be premalignant lesion, others suggest that it more display molecular pathogenesis of epithelial rather than dysplastic epithelium.³

The term dysplasia normally associated with pre-malignant condition that refer to an abnormality of development or an epithelial anomaly of growth and differentiation. Histopathological changes of dysplastic epithelial cells are similar to squamous cell carcinoma (SCC). Dysplasia is further categorized into three groups mild, moderate, and severe – depending on the degree of involvement of the epithelial.^{4,5}

Squamous cell carcinoma (SCC) accounts for approximately 94% of oral and oropharyngeal malignancies.³ In Iran SCC rates are 1.5 times higher in men than women and the increase becomes more rapid in the second half of life.⁶ SCC arise from the epithelial dysplasia and spread into or invade the underlying tissues. Keratin pearl (concentric layers of keratinized cells) is found in regions where abnormal squamous cells form concentric layers (epithelial island). Histopathologic grading of tumors has been used for many years to predict the outcome of a tumor, although with varying prognostic value. Many systems of grading epithelial dysplasia have been proposed in order to standardize the severity of dysplastic features. In addition, the parameters considered in the histological assessment should be biologically meaningful, reflecting the malignant potential of the lesion.³

The syndecans are a gene family of four transmembrane heparan sulfate proteoglycans associated with the cell surface and extracellular matrix and consist of a protein core to which heparan sulfate chains are covalently attached. The syndecan family consists of four members, syndecan-1 (CD138) - Syndecan-4, each encoded by distinct genes. The syndecan-1 protein functions as an integral membrane protein and participates in cell proliferation, cell migration and cell-matrix interactions via its receptor for extracellular matrix proteins. Syndecan-1 via heparan sulfate chains can bind to interstitial matrix including types I, III, V collagen, fibrillar collagen, elastin, and fibronectin, as well as can mediate both cell-cell and cell-extracellular matrix interactions.

Several studies on head and neck carcinoma have suggested that reduced expression of syndecan-1 is associated with the prognosis of such neoplasms.⁸ however; syndecan-1 is up-regulated in breast and liver cancers.⁹ Therefore, the relationship between cancer and syndecan-1 expression is still unclear. It may depend on the location of cancer and influence of other cells or proteins, or some other factors.^{10,11}

In literature, very less information is available on comparative account of expression of syndecan-1 in

erosive lichen planus, epithelial dysplasia, and OSCC; therefore, this study was undertaken to evaluate the expression of syndecan-1 in erosive lichen planus, epithelial dysplasia, and OSCC.

Materials and Methods

The present study was conducted on 35 paraffin blocks (paraffin –embedded) including 5 cases of dysplasia, 10 cases of lichen planus (erosive and without dysplasia) and 15 cases of OSCC. The Blocks were retrieved from Archives of Pathology (2009-2015), School of Dental Medicine, Ahvaz Jundishapur University of medical sciences. Two sections of 4μ(M) each from the paraffin blocks were made. The first section was examined by pathologists to confirm the diagnosis. The second section was mounted on poly-L-lysine-coated glass slides. The slides were air-dried at 37°C for 2h on a heating plate. The samples from each block were deparaffinized with xylene and then were rehydrated through graded ethanol to distilled water.

In order to block endogenous peroxidase activity the samples were incubated with 3% H₂O₂ for 30 minutes at room temperature. The cells were then washed with buffered saline solution (PH=7.2). The Immunohistochemical staining (Abcam, England) for syndecan-1 was performed according to the manufacturer's recommendations.

After incubation with primary antibody the EnVision technique was used. In the next step positive antigen-antibody reactions were visualized by incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB, DAKO). The sections were counterstained with hematoxylin and after dewatering were mounted on plates. Finally, the immunohistochemical staining intensity was evaluated independently under a light microscope (CX21; Olympus Corporation, Tokyo, Japan) by pathologist.

In this study, normal epithelial tissue was applied as positive control and no primary antibody was used as negative controls. Staining intensity was divided into categories of mild (light brown), moderate (brown), and severe (dark brown). The samples were classified according to marker location. Localization of staining was predominantly membranous and cytoplasmic membrane. Data were analyzed using chi-square tests and SPSS version 20.

Results

The quantitative assessment of immunohistochemical staining intensity for syndecan-1 was performed. Following data collection statistical data analysis was performed.

Table 1.The staining intensity of study group

Group		Staining intensity			Total
		Mild	Moderate	Severe	
Squamous cell carcinoma	Number	10	9	1	20
	Percentage (%)	50.0%	45.0%	5.0%	100.0%
Lichen planus	Number	1	4	5	10
	Percentage (%)	10.0%	40.0%	50.0%	100.0%
Dysplasia	Number	0	1	4	5
	Percentage (%)	0.0%	20.0%	80.0%	100.0%
Total	Number	11	14	10	35
	Percentage (%)	31.4%	40.0%	28.6%	100.0%

Most of the OSCC samples had the mild and moderate staining pattern. Moderately differentiated samples had lower staining intensity (Fig.1). Well-differentiated samples showed high staining intensity, but in the foci of squamous epithelium with marked keratinization no staining was observed which was similar to normal epithelium (Fig.2). The staining pattern of lichen planus samples staining were often moderate and severe (Fig. 3) and the staining of dysplasia samples were severe similar to

normal epithelium. Similarly, the normal epithelium showed severe staining and the epithelial appendages such as sebaceous glands, hair follicles, and gland ducts were also showed staining (Fig. 4). The chi-square analysis of lesions at different levels showed different staining intensity ($\chi^2=15.97$, $df=4$, $P\text{-Value}=0.03$) and the rate of intensity of staining decreased from lichen, dysplasia to OSCC, respectively.

Table 2.The profile of staining according to location

Group		Profile of staining according to location		Total
		Membrane	<i>cytoplasmic membrane</i>	
Squamous cell carcinoma	Number	2	18	20
	Percentage (%)	10.0%	90.0%	100.0%
Lichen planus	Number	8	2	10
	Percentage (%)	80.0%	20.0%	100.0%
Dysplasia	Number	5	0	5
	Percentage (%)	100.0%	0.0%	100.0%
Total	Number	15	20	35
	Percentage (%)	42.9%	57.1%	100.0%

The marker expression was different in three lesions. In most cases of OSCC staining was more appeared in membranous – cytoplasmic site, but membrane staining was more found in lichen planus and dysplasia; however, in areas with severe dysplasia membranous-cytoplasmic staining was observed (Fig.

5). Data analyses revealed a significant correlation between lesions and marker expressions ($\chi^2=21.117$, $df=2$, $P\text{-Value}=0.00$). The rate of staining was found more in cytoplasmic membrane and the range of variation increased from dysplasia to lichen planus and OSCC, respectively.

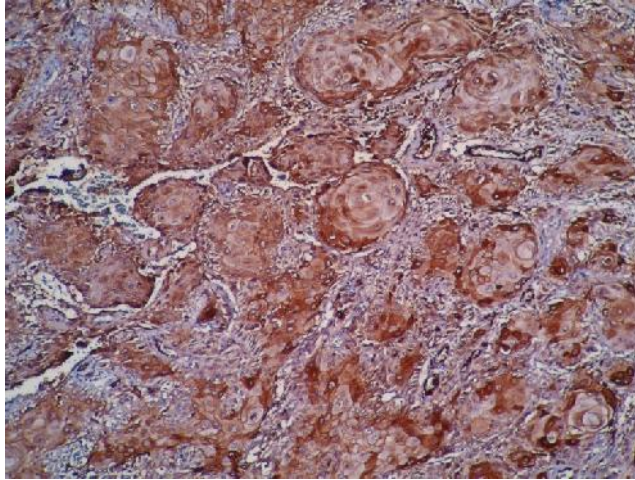


Figure1: Moderately differentiated OSCC: almost all of squamous cell have moderate and often membranous–cytoplasmicstaining pattern (100 x magnification)

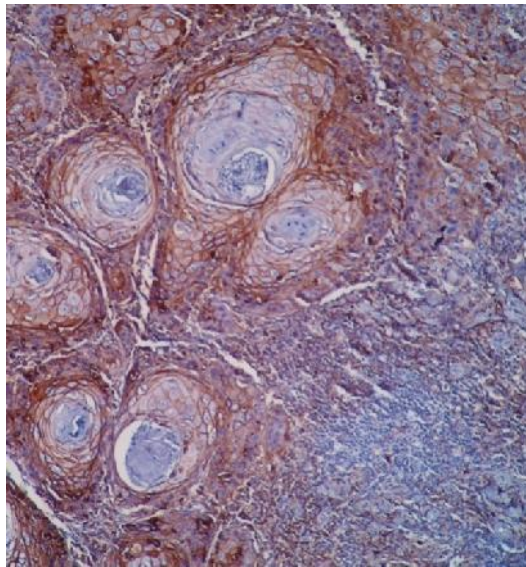


Figure 2: well-differentiated OSCC with severe and membranous staining pattern (100 x magnification).

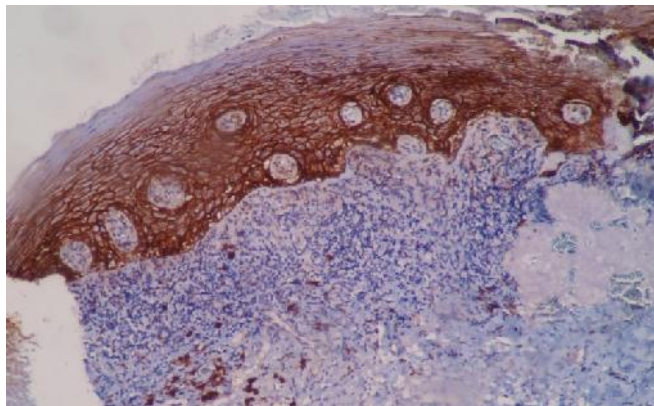


Figure 3: Lichen planus with severe and more membranous staining pattern and negative staining of lymphocytes (100 x magnifications).

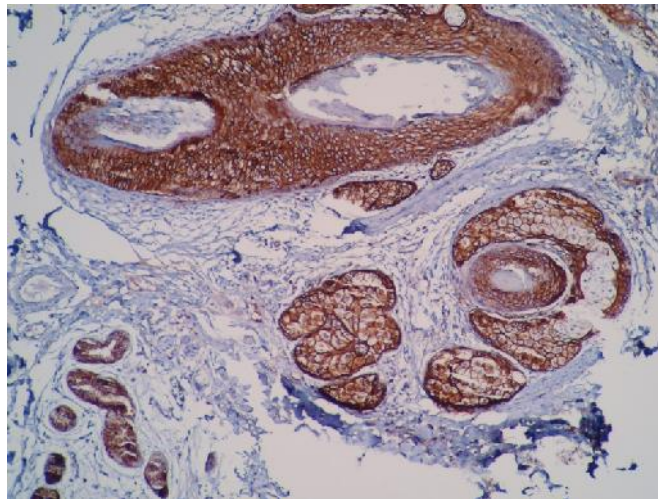


Figure 4: staining in epithelial appendages such as hair follicles, sebaceous glands and gland ducts (magnification 100 x).

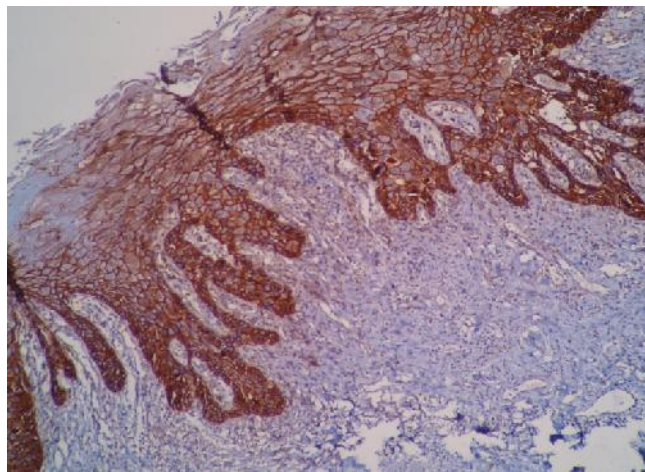


Figure 5: mild dysplasia with severe and often membranous staining pattern (magnification 100 x).

Discussion

In the present study, a total of 20 samples of OSCC, 10 samples of lichen planus, and 5 samples of dysplasia were examined. The staining patterns and intensity observed in OSCC samples in most cases was mild (50%) or moderate (45%) and only one case (5%) showed severe staining. In lichen planus the staining intensity pattern in most cases was severe (50%) or moderate (40%) and only one case (10%) showed mild staining. Similarly, the samples of dysplasia showed 4 cases (80%) of severe staining, one case (20%) of moderate staining and none of the samples had mild staining.

Two stained samples (10%) in OSCC had membranous staining and 18 samples (90%) were stained both in the membrane and cytoplasm surface, but all of the dysplasia samples (100%) had membranous staining.

According to result of present study high-grade staining of dysplastic was more detected compared two other groups and was similar to positive tissue control (normal epithelium). As well as, staining intensity in three layers of epithelium (stratum basale, stratum spinosum, and superficial layer) was evaluated.

The syndecan-1 expression in basal layer with mild intensity was observed in all dysplasia samples and normal epithelium tissue while no syndecan-1 expression was found in superficial layers and different intensities was just seen in the stratum spinosum staining.

Lakkam *et al.*, 2014 in a study showed that all samples of normal mucosa had severe staining for syndecan-1 whereas 19 cases (47.5%) of dysplasia had mild staining, 9 cases (22.5%) moderate staining, and 12 cases (30%) showed severe staining and concluded that the reduction of syndecan-1 expression is associated with dysplastic oral epithelium. It is noteworthy that in some cases there were no evidence for interference of hematoxylin and eosin staining in dysplastic oral epithelium, but reduction of syndecan-1 expression can be a sign of dysplastic variations.¹²

In the present study, syndecan-1 expression was reduced in OSCC compared to dysplasia, but since the studied samples were mostly mild to moderate dysplasia there was no possibility of comparing mild to severe dysplasia. Additionally, the similarity of marker expression to normal tissue possibly was due to mildness of the vast majority of dysplasia cases.

As well as, in another study Sushant *et al.*, 2013 concluded that there was no significant difference in staining intensity of syndecan-1 expression between normal epithelium and mild dysplasia which was consistent with the result of the present study. The results of Sushant *et al.*, 2013 and Kurokawa *et al.*, 2003 showed that the expression of syndecan-1 decreased with increasing grades of dysplasia, therefore the marker can be a prognostic factor in the clinical assessment.^{13,14} The other similarity between Sushant study and present study was the various expression of syndecan-1 in just the stratum spinosum. The stratum basale, and superficial layer both had similar expression.¹³

Oral epithelial dysplasia is a predictor term used to describe the histopathological changes seen in a chronic, progressive and premalignant disorder of the oral mucosa and the likelihood of the development to carcinoma is related to the increased degree of dysplasia.¹⁴ The malignancy development is associated with the loss of cell-cell association, loss of differentiation and cellular changes in epithelial layer.¹³ Therefore, the diagnosis of dysplasia is very important. The severity-based diagnostic criteria for dysplasia can vary by different pathologist¹⁴ and cellular changes expected in the dysplasia may clearly be seen in H & E stained samples.¹² This suggests that syndecan-1 expression may be one of the most important marker to determine the degree of dysplasia¹⁴ and reduced severity of this marker can be alarming.¹³

In addition to the staining intensity, the location of marker expression were also studied and it was found that the syndecan-1 expression was located only at

the cell membrane and associated with dysplastic change in the oral epithelium.

According to the results of present study the immunohistochemical staining of OSCC in most of the cases showed mild to moderate staining. The well-differentiated cases had more staining intensity than moderately differentiated cases.

Ro *et al.*, 2006 in a study observed that the reduction of syndecan-1 expression was closely associated with poorly differentiated SCC of tongue and well and moderately differentiated cases had more staining intensity.¹¹ Similarly, Soukka *et al.*'s study showed that syndecan-1 was reduced along with increase in the degree of dysplasia and poorly differentiated SCC had slightly lower percentages of positive staining compared to well differentiated and moderately differentiated SCC.¹⁵

Moreover, in a study by Inki *et al.*, 1996 only 21% of SCC showed staining similar to normal tissue, and other cases had less staining intensity.¹⁰

In summary, in the present study only 5% of SCC group showed severe staining and Ro *et al.*, Soukka *et al.*, and Inki *et al.*'s study have been reported the SCC staining 22%, 35% and 21%, respectively. Although the overall percentage of studies are somewhat different, but all showed reduced expression of markers in the SCC.

Furthermore, it was observed that cells with morphologically better differentiation had staining patterns similar to normal epithelium (severe staining). As well as, the location of markers were examined and it was found that most of the OSCC had staining in both membrane and cytoplasm, but better differentiated cell revealed membranous pattern of staining.

Two hypotheses can be advanced to explain the discrepancy: 1- poorly differentiated cells have been associated with the formation of the imperfect marker and cannot appear in the membrane and so cytoplasmic membrane surrounds all cells in nature. 2- due to the deficiency of protein the cells attempts to build higher levels of this protein that in addition to being in the membrane also accumulate in the cytoplasm, but since differ from normal syndecan-1, does not meet cell efficiency criteria (e.g., do not maintain cell-cell or cell-matrix adhesions). According to the present study, lower expression was found in severe dysplasia and the expression was localized in the membrane, but cytoplasmic localization was evident in the well differentiated cells.

In general, morphological changes are considered a hallmark of malignancies which lead to cell adhesion variations. Loss of syndecan-1 expression in tumor cells leads to decreased cell-cell adhesion and extracellular matrix and do not respond to signals sent from the surrounding cell. Thereby, reduced expression of this marker can be alarming.¹³

Zyada *et al.*'s study in 2010 was the only study which examined the effect of syndecan-1 expression on lichen planus, the result showed that reduction of syndecan-1 expression is associated with dysplastic change rate in cases of oral lichen planus.

The aim of this study was to determine the syndecan-1 expression in erosive lichen planus, epithelial dysplasia, and OSCC. According to the findings of previous studies it is assumed that the level of expression in lichen planus is similar to normal tissue and in dysplasia less than normal tissue, and finally SCC is less than all. The result of dysplasia and SCC in this study was similar to what was expected, but the expression of syndecan-1 in lichen planus was greater than the dysplasia.

What is more notably important is the location of marker expression. Two cases of lichen planus similar to SCCs were localized both in cytoplasm and membrane. This could indicate similar molecular changes similar to malignant and premalignant potential of some lichen planus. The results support the premalignant potential for lichen planus, but this requires further investigation.

Conclusion

The expression of syndecan-1 in SCC was significantly less than lichen planus and dysplasia. The reduction of the expression of syndecan-1 in Lichen Planus (similar to what was seen in OSCC) may be a sign of a potential pre-malignant of lesion, but this requires further investigation.

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